Data Evaluation Report on the Acute Toxicity of Ethylenethiourea to Fish (*Lepomis macrochirus*)

PMRA Submission Number N/A EPA MRID Number 47441202

Data Requirement:	PMRA DATA CODE	{.....}
	EPA DP Barcode	D353731
	OECD Data Point	{.....}
	EPA MRID	47441202
	EPA Guideline	OPPTS 850.1075 (72-1)

Test material: Ethylenethiourea; 2-imidazolidinethione **Purity:** 100%
Common name: Ethylenethiourea
Chemical name: IUPAC: Not Reported
CAS name: Not Reported
CAS No.: 96-45-7 (2-imidazolidinethione)
Synonyms: None Reported

Primary Reviewer: John Marton **Signature:**
Staff Scientist, Cambridge Environmental, Inc. **Date:** 07/22/08

Secondary Reviewer: Teri S. Myers **Signature:**
Senior Scientist, Cambridge Environmental, Inc. **Date:** 03/03/09

Primary Reviewer: Brian Montague, Fishery Biologist USEPA **Date:** February 2015

Secondary Reviewer(s): Allen W. Vaughan **Date:** 04/16/15

Reference/Submission No.: {.....}

Company Code	{.....}	[For PMRA]
Active Code	{.....}	[For PMRA]
Use Site Category:	{.....}	[For PMRA]
EPA PC Code	600016	

Date Evaluation Completed: 11-02-15

CITATION: Soucy, K. 2008. Ethylenethiourea- Acute Toxicity to Bluegill Sunfish (*Lepomis macrochirus*) Under Static Conditions, Following OPPTS Draft Guideline 850.1075. Unpublished study performed by Springborn Smithers Laboratories, 790 Main St., Wareham, Massachusetts 02571-1037. Laboratory report number 13921.6104. Study submitted to EBDC/ETU Task Force, c/o McDermott, Will and Emery LLP, 600 13th St NW, Washington, DC 20005. Study submitted June 2, 2008.

DISCLAIMER: This document provides guidance for EPA and PMRA reviewers on how to complete a data evaluation record after reviewing a scientific study concerning the acute toxicity of a pesticide to fish. It is not intended to prescribe conditions to any external party for conducting this study nor to establish absolute criteria regarding the assessment of whether the study is scientifically sound and whether the study satisfies any applicable data requirements. Reviewers are expected to review and to determine for each study, on a case-by-case basis, whether it is scientifically sound and provides sufficient information to satisfy applicable data requirements. Studies that fail to meet any of the conditions may be accepted, if appropriate; similarly, studies that meet all of the conditions may be rejected, if appropriate. In sum, the reviewer is to take into account the totality of factors related to the test methodology and results in determining the acceptability of the study.

EXECUTIVE SUMMARY:

In a 96-h acute toxicity study, bluegill sunfish (*Lepomis macrochirus*) were exposed to ethylenethiorurea at nominal concentrations of 0 (negative control), 63, 130, 250, 500 and 1000 mg ai/L under static conditions. The 96-hour reviewer-calculated time-weighted average (TWA) concentrations were <4.5 (<LOQ; control), 60, 123, 243, 470 and 988 mg ai/L. The 96-h LC_{50} was >988 mg ai/L. No mortality or sub-lethal effects were observed in the control or any of the ethylenethiourea treatment groups, yielding EC_{50} and NOAEC values of >988 and 988 mg ai/L, respectively. Based on the results of this study, ethylenethiourea would be classified as practically non-toxic to bluegill sunfish in accordance with the classification system of the U.S. EPA

This toxicity study is classified as acceptable and satisfies the guideline requirement for an acute freshwater fish toxicity study.

Results Synopsis

Test Organism Size/Age(mean weight or length): Wet Weight- 0.43 (0.29-0.69) g; Length- 31 (27-36) mm
Test Type (Flow-through, Static, Static Renewal): Static

LC_{50}: >988 mg ai/L 95% C.I.: N/A
NOAEC: 988 mg ai/L Probit Slope: N/A
EC_{50}: >988 mg ai/L 95% C.I.: N/A
Endpoint(s) Affected: None

I. MATERIALS AND METHODS

GUIDELINE FOLLOWED: This study was conducted following guidelines outlined in the U.S. Environmental Protection Agency's Ecological Effects Test Guideline (draft) OPPTS 850.1075- Fish, Acute Toxicity Test, Freshwater and Marine (U.S. EPA, 1996). The following deviation from OPPTS 850.1075 was noted:

The hardness of the dilution water (38-44 mg/L as $CaCO_3$) was slightly outside the recommended hardness range (40-180 mg/L as $CaCO_3$).

This deviation did not appear to impact the results of the study.

COMPLIANCE: Signed and dated No Data Confidentiality, GLP and Quality Assurance statements were provided. This study was conducted in compliance with all pertinent U.S. EPA Good Laboratory Practice regulations (40 CFR, Part 160) with the following exceptions: routine dilution water and food contaminant screening analyses for pesticides, PCBs and toxic metals were conducted at GeoLabs, Inc., Braintree, Massachusetts using standard U.S. EPA Procedures and are considered facility records. Since the analyses were conducted following standard validated methods, these exceptions had no impact on the study results.

A. MATERIALS:

1. Test material Ethylenethiourea

Description: Solid

Lot No./Batch No. : 04816CH (Lot No.)

Purity: 100%

Stability of compound under test conditions: Stable. Method validation was conducted by determining the recovery of ethylenethiourea from 0.1% trifluoroacetic acid in 20 ppt filtered seawater at fortified concentrations of 0, 0.300, 20.0 and 1000 mg/L. Individual recoveries ranged from 74.7 to 103% of nominal with a mean recovery of 93.4%.

(OECD recommends water solubility, stability in water and light, pKa, Pow, and vapor pressure of test compound)

Storage conditions of test chemicals: Stored in the dark at ambient temperature

Physicochemical properties of ethylenethiourea.

Parameter	Values	Comments
Water solubility at 20°C	Not Reported	

Parameter	Values	Comments
Vapor pressure	Not Reported	
UV absorption	Not Reported	
pKa	Not Reported	
Kow	Not Reported	

2. Test organism:

Species: Bluegill Sunfish (*Lepomis macrochirus*)
Age at test initiation: Not Reported
Weight at study initiation: 0.43 (0.29-0.69) g; N = 30
Length at study initiation: 31 (27-36) mm; N = 30
Source: Osage Catfisheries, Qsage Beach, Missouri

B. STUDY DESIGN:

1. Experimental Conditions

a. Range-finding study: A static 96-hour range-finding study was conducted by exposing 5 fish/level to nominal concentrations of 0 (negative control), 0.010, 0.10, 1.0, 10 and 100 mg ai/L. At test termination, no mortality or adverse effects were observed in the control or treatment levels. Based on the results and consultation with the Study Sponsor, nominal concentrations of 63, 130, 250, 500 and 1000 mg ai/L were chosen for the definitive exposure.

b. Definitive Study

Table 1: Experimental Parameters

Parameter	Details	Remarks / *Criteria*
Acclimation		
Period:	14 Days	*The recommended acclimation period is a minimum of 14 days; OECD guideline recommends a minimum of 12 days. Pretest mortality should be < 3% 48 h. prior to testing. OECD pretest mortality criteria: >10% = rejection of entire batch; ≥ 5 and ≤ 10% = continued acclimation for 7 days; <5% = acceptable.*
Conditions: (same as test or not)	Same as test	
Feeding:	The fish were fed Ziegler Brothers Prime Fish Flakes, *ad libitum*, at least once daily. Fish were not fed during the 48 hours prior to test initiation.	
Health: (any mortality observed)	No mortalities were observed during the 7 days prior to testing.	
Duration of the test	96 hours	*The recommended test duration is 96 hours.*

Parameter	Details	Remarks *Criteria*
<u>Test condition</u> Static/flow-through Type of dilution system - for flow-through method. Renewal rate for static renewal	 Static N/A N/A	*A reproducible supply of toxicant is recommended. Consistent flow rate is usually 5-10 vol/24 hours; meter systems should be calibrated before and after study and checked twice daily during test period.*
Aeration, if any	None	*Aeration is not recommended; OECD guideline recommends aeration. If aeration is necessary, test solutions must be analyzed periodically to verify exposure.*
<u>Test vessel</u> Material: (glass/stainless steel) Size: Fill volume:	 Glass 39 x 20 x 25 cm 15 L	*Test vessel size is usually 19 L (5 gal) or 30 x 60 x 30 cm.* *Fill volume is usually 15-30 L of solution.*
Source of dilution water Quality:	The dilution water was a combination of unchlorinated Town of Wareham well water and Springborn Smithers well water. Several species of daphnids are maintained in water from the same source as the dilution water utilized in this study and have successfully survived and reproduced over multiple generations.	*Recommended source of dilution water is soft, reconstituted water or water from a natural source. EPA does not recommend the use of dechlorinated tap water; however, its use may be supportable if the biological responses for the organisms and chemical analyses of residual chlorine meet conditions in the Agency guidelines for dilution water Dilution water should be intensely aerated before the study.* *OECD permits dechlorinated tap water.*

Parameter	Details	Remarks *Criteria*
Water parameters: Hardness pH Dissolved oxygen Total Organic carbon Particulate Matter Metals Pesticides Chlorine Temperature {Salinity for marine or estuarine species} Intervals of water quality measurement	 38-44 mg/L as $CaCO_3$ 6.6-7.9 6.6-9.3 mg/L; >60% saturation 0.98 mg/L (March 2008) Not Reported Not Reported Not Reported Not Reported 21-22°C N/A Temperature, DO and pH were measured daily in each replicate test vessel. Additionally, temperature was continuously measured in replicate A of the nominal 1000 mg ai/L treatment level.	 *Hardness:* *EPA recommends 40 - 48 mg/L as $CaCO_3$ (OECD recommends 10 - 250 mg/L)* *pH:* *EPA recommends* *(OECD recommends pH 6.0 - 8.5)* *Dissolved Oxygen:* *EPA recommends: Static:>60% during first 48 hrs and >40% during second 48 hrs; flow-through: > 60%; (OECD guideline recommends at least 80% saturation value).* *Temperature:* *EPA recommends 12 C for coldwater species, 17 or 22 C for warmwater species, and 22 ± 1 C for estuarine/marine organisms. (OECD recommends 21 - 25°C for bluegill and 13 - 17°C for rainbow trout).* *Salinity*: N.A. *Water quality should be measured at beginning of test and every 48 hours.*
Number of replicates/groups: control: solvent control: treated ones:	 2 N/A 2/level	*Recommended number of replicates include a control and five treatment levels. Each concentration should be 60% of the next highest concentration; concentrations should be in a geo-metric series.*
Number of organisms per replicate /groups: control: solvent control: treated ones:	 10 N/A 10	*Number of organisms per replicate should be ∃10/concentration; OECD guideline recommends at least 7 fish/concentration.*

Parameter	Details	Remarks *Criteria*
Biomass loading rate	0.29 g/L	*Recommended static conditions are # 0.8 g/L at #17EC and #0.5 g/L at > 17EC. Recommended flow-through conditions are #1 g/L/day. OECD recommends a maximum of 1 g fish/L for static and semi-static, while higher rates are recommended for flow-through.*
<u>Test concentrations:</u> nominal: measured:	0 (negative control), 63, 130, 250, 500 and 1000 mg ai/L <4.5 (<LOQ; control), 60, 123, 243, 470 and 988 mg ai/L	The measured concentrations represent the reviewer-calculated TWA concentrations. The mean-measured concentrations were 60, 120, 240, 470 and 990 mg ai/L.
Solvent (type, percentage, if used)	N/A; a solvent was not used	*The solvent should not exceed 0.5 ml/L for static tests or 0.1 ml/L for flow-through tests; OECD recommends that the solvent not exceed 100 mg/L.*
Lighting	16L:8D; sudden transitions from light to dark were avoided. The test area was illuminated with fluorescent bulbs at an intensity range of 33-50 footcandles (360-540lux) at the solutions' surface.	*The recommended photo period is 16 hours of light and 8 hours of dark with a 15-30 minute transition period. OECD recommends a photo period of 12 -16 hours.*
Feeding	Fish were not fed during the test.	*Fish should not feed during the study.*
<u>Recovery of chemical</u> Frequency of determination Level of quantization Level of detection	 Samples were collected at 0, 48 and 96 hours 3.8-4.8 mg ai/L Not Reported	
Positive control {if used, indicate the chemical and concentrations}	N/A; a positive control was not used	
Other parameters, if any	None	

2. Observations:

Table 2: Observations

Parameter	Details	Remarks *Criteria*
Parameters measured including the sublethal effects/toxicity symptoms	-Mortality -Sub-Lethal Effects	Death was defined as the lack of movement by the exposed organisms (i.e., absence of gill movement and reaction to gentle prodding).
Observation intervals	0, 6, 24, 48, 72 and 96 hours	*Observation intervals should be a minimum of every 24 hours.*
Were raw data included?	No, summarized treatment level data were provided. These data were adequate for statistical analyses.	
Other observations, if any	All test solutions appeared clear and colorless without any undissolved test material.	

II. RESULTS AND DISCUSSION:

A. MORTALITY:

No mortalities were observed in the control or treatment groups at any point during the 96-hour exposure period, yielding NOAEC and LC_{50} values of 990 and >990 mg ai/L, respectively, based on the mean-measured concentrations. The NOAEC and LC_{50} values were 988 and >988 mg ai/L, respectively, based on the reviewer-calculated TWA concentrations.

Table 3: Effect of Ethylenethiourea on Mortality of *Lepomis macrochirus*.

TWA and (Nominal) Concentrations mg ai/L	No. of Fish at Start of Study	Observation Period					
		Day 1		Day 3		Day 4	
		No Dead	% Mortality	No Dead	% Mortality	No Dead	% Mortality
Negative Control	20	0	0	0	0	0	0
60 (63)	20	0	0	0	0	0	0
123 (130)	20	0	0	0	0	0	0
243 (250)	20	0	0	0	0	0	0
470 (500)	20	0	0	0	0	0	0
988 (1000)	20	0	0	0	0	0	0
NOAEC[1]	988 mg ai/L						
LC_{50}[1]	>988 mg ai/L						
Positive control, if used mortality: LC_{50}:	N/A	N/A	N/A	N/A	N/A	N/A	N/A

[1] Values based on the reviewer-calculated TWA concentrations
N/A- Not Applicable

B. NON-LETHAL TOXICITY ENDPOINTS:

All surviving fish appeared normal and healthy throughout the 96-hour exposure period, yielding NOAEC and EC_{50} values of 990 and >990 mg ai/L, respectively, based on the mean-measured concentrations. The NOAEC and EC_{50} values were 988 and >988 mg ai/L, respectively, based on the reviewer-calculated TWA concentrations.

Table 4: Sub-lethal Effect of Ethylenethiourea on *Cyprinodon variegatus*.

TWA and (Nominal) Concentrations mg ai/L	Observation Period		
	Endpoints at Day 1	Endpoints at Day 3	Endpoints at Day 4
	% Affected	% Affected	% Affected
Negative Control	A.N.	A.N.	A.N.
60 (63)	A.N.	A.N.	A.N.
123 (130)	A.N.	A.N.	A.N.
243 (250)	A.N.	A.N.	A.N.
470 (500)	A.N.	A.N.	A.N.
988 (1000)	A.N.	A.N.	A.N.
NOAEC[1]	988 mg ai/L		
LOAEC[1]	>988 mg ai/L		
EC_{50}[1]	>988 mg ai/L		
Positive control, if used % sublethal effect: EC_{50}:	N/A	N/A	N/A

[1] Values based on the reviewer-calculated TWA concentrations
A.N.- All surviving fish appear normal and healthy
N/A- Not Applicable

C. REPORTED STATISTICS:

During this study, no concentration tested resulted in ≥50% mortality, therefore, the LC_{50} value was empirically estimated to be greater than the highest mean-measured concentration tested. The NOAEC value was determined using Kruskal-Wallis' Test. Data were first checked for normality using Chi-Square Test (Weber et al., 1989) and for homogeneity of variance using Bartlett's Test (Horning and Weber, 1985). These statistical determinations were performed using TOXSTAT® Version 3.5 (Gulley et al., 1996).

D. VERIFICATION OF STATISTICAL RESULTS:

Statistical Method: The lack of mortality and sub-lethal effects precluded the statistical analysis of the data. All toxicity vales were visually determined based on the reviewer-calculated TWA concentrations.

LC_{50}: >988 mg ai/L 95% C.I.: N/A
EC_{50}: >988 mg ai/L 95% C.I.: N/A
NOAEC: 988 mg ai/L
Probit Slope: N/A 95% C.I.: N/A

E. STUDY DEFICIENCIES:

There were no study deficiencies.

F. REVIEWER'S COMMENTS:

The reviewer's results were similar to those of the study author with the exception that the reviewer reported all toxicity values based on the TWA concentrations, while the study author reported all toxicity values based on the mean-measured concentrations. Therefore, the reviewer's results are reported in the Executive Summary and Conclusions sections of this DER.

The reviewer calculated the TWA concentrations using the following equation:

$$C_{TWA} = \frac{\left(\frac{C_1 + C_0}{2}\right)(t_1 - t_0) + \left(\frac{C_2 + C_1}{2}\right)(t_2 - t_1) + \left(\frac{C_{n-1} + C_2}{2}\right)(t_{n-1} - t_2) + \left(\frac{C_n + C_{n-1}}{2}\right)(t_n - t_{n-1})}{t_n}$$

where:
C TWA is the time-weighted average concentration,
C j is the concentration measured at time interval j (j = 0, 1, 2,...n)
t j is the number of hours (or days or weeks, units used just need to be consistent in the equation) of the test at time interval j
(e.g., t 0 = 0 hours (test initiation), t 1 =24 hours, t 2 =96 hours)

Analysis of the three QC samples yielded recoveries of 79.6-98.8% of nominal. The 79.6% recovery occurred at test initiation at the 1000 mg ai/L QC sample and this recovery was outside of the acceptable range (80-120% of nominal). However, the recovery at this level was 93.2% at 48 hours and 90.9% at 96 hours.

The initial measured concentrations, obtained from the samples collected from the test vessels, yielded recoveries of 88.9-95.0% of nominal. Recoveries at 48 hours were 100.0-107.1% of initially measured concentrations, while measured concentrations at test termination were 105.3-113.0% of initial measured concentrations. The 96-hour TWA concentrations yielded recoveries of 94.2-98.8% of nominal.

The parent compounds of the test material were [[1,2-ethanediylbis[carbamodithioato]](2-) manganese mixture with [[1,2-ethandiylbis[carbamodithioato]](2-)zinc.

The in-life portion of the definitive toxicity test was conducted from March 24 to March 28, 2008.

G. CONCLUSIONS:

This study is scientifically sound and acceptable for use in Agency risk assessments. The NOAEC, LC_{50} and EC_{50} values, based on a lack of mortality and sub-lethal effects, were 988, >988 and >988 mg ai/L, respectively.

LC_{50}: >988 mg ai/L 95% C.I.: N/A
EC_{50}: >988 mg ai/L 95% C.I.: N/A
NOAEC: 988 mg ai/L
Probit Slope: N/A 95% C.I.: N/A

III. REFERENCES:

ASTM. 2002. Standard practice for conducting acute toxicity tests with fishes, macroinvertebrates and amphibians. Standard E729-96. American Society for Testing and Materials, 100 Barr Harbor Road, West Conshohocken, PA 19428.

Gulley, D.D., Boelter, A.M. and Bergman, H.L. 1996 TOXSTAT® Release 3.5. University of Wyoming, Laramie, Wyoming.

Horning, W.B. and C.I. Weber. 1985. Short-term methods for estimating the chronic toxicity of effluents and receiving waters to freshwater organisms. EPA/600/4-85/014. Environmental Monitoring and Support Laboratory, U.S. Environmental Protection Agency, Cincinnati, Ohio.

U.S. EPA. Federal Insecticide, Fungicide and Rodenticide Act (FIFRA); Good Laboratory Practice Standards; Final Rule (40 CFR, Part 160). U.S. Environmental Protection Agency, Washington, DC.

U.S. EPA. 1996. Office of Prevention, Pesticides and Toxic Substances. Ecological Effects Guideline, OPPTS 850.1075. Fish Acute Toxicity Test, Freshwater and Marine. "Public Draft". EPA 712-C-96-118. April 1996. U.S. Environmental Protection Agency, Washington, DC.

Weber, C.I., W.H. Peltier, T.J. Norberg-King, W.B. Horning II, F.A. Kessler, J.R. Menkedick, T.W. Heiheisel, P.A. Lewis, D.J. Klemm, Q.H. Pickering, E.L. Robinson, J.M. Lazorchak, L.J. Wymer and R.W. Freyberg (eds.). 1989. Short-term methods for estimating the chronic toxicity of effluents and receiving waters to freshwater organisms. 2nd ed. EPA/600/4/89/001. Environmental Monitoring Systems Laboratory, U.S. Environmental Protection Agency, Cincinnati, OH.